

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding all or a substantial portion of a dihydroxyacid dehydratase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

2. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

3. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.

4. A transformed host cell comprising the chimeric gene of Claim 3.

5. A dihydroxyacid dehydratase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6.

6. An isolated nucleic acid fragment encoding all or a substantial portion of a branched chain amino acid aminotransferase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:31, and SEQ ID NO:33;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:31, and SEQ ID NO:33; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

7. The isolated nucleic acid fragment of Claim 6 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:30, and SEQ ID NO:32.

8. A chimeric gene comprising the nucleic acid fragment of Claim 6 operably linked to suitable regulatory sequences.

9. A transformed host cell comprising the chimeric gene of Claim 8.

10. A branched chain amino acid amino transferase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:31, and SEQ ID NO:33.

11. An isolated nucleic acid fragment encoding all or a substantial portion of a *leuC* subunit of 3-isopropylmalate dehydratase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, and SEQ ID NO:44;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, and SEQ ID NO:44; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

12. The isolated nucleic acid fragment of Claim 11 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, and SEQ ID NO:43.

13. A chimeric gene comprising the nucleic acid fragment of Claim 11 operably linked to suitable regulatory sequences.

14. A transformed host cell comprising the chimeric gene of Claim 13.

15. A *leuC* subunit of 3-isopropylmalate dehydratase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, and SEQ ID NO:44.

16. An isolated nucleic acid fragment encoding all or a substantial portion of a *leuD* subunit of 3-isopropylmalate dehydratase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, and SEQ ID NO:53;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, and SEQ ID NO:53; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

17. The isolated nucleic acid fragment of Claim 16 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, and SEQ ID NO:52.

18. A chimeric gene comprising the nucleic acid fragment of Claim 16 operably linked to suitable regulatory sequences.

19. A transformed host cell comprising the chimeric gene of Claim 18.

20. A *leuD* subunit of 3-isopropylmalate dehydratase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, and SEQ ID NO:53.

21. A method of altering the level of expression of a branched chain amino acid biosynthetic enzyme in a host cell comprising:

- (a) transforming a host cell with the chimeric gene of any of Claims 3, 8, 13, and 18; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of a branched chain amino acid biosynthetic enzyme in the transformed host cell.

22. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a branched chain amino acid biosynthetic enzyme comprising:

- (a) probing a cDNA or genomic library with the nucleic acid fragment of any of Claims 1, 6, 11, and 16;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of any of Claims 1, 6, 11, and 16;

- (c) isolating the DNA clone identified in step (b); and
(d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a branched chain amino acid biosynthetic enzyme.

23. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a branched chain amino acid biosynthetic enzyme comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 5, 8, 10, 12, 14, 16, 18, 21, 23, 25, 27, 30, 32, 35, 37, 39, 41, 43, 46, 48, 50, and 51; and
(b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a branched chain amino acid biosynthetic enzyme.

24. The product of the method of Claim 22.

25. The product of the method of Claim 23.

26. A method for evaluating at least one compound for its ability to inhibit the activity of a branched chain amino acid biosynthetic enzyme, the method comprising the steps of:

- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a branched chain amino acid biosynthetic enzyme, operably linked to suitable regulatory sequences;
(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the branched chain amino acid biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;
(c) optionally purifying the branched chain amino acid biosynthetic enzyme expressed by the transformed host cell;
(d) treating the branched chain amino acid biosynthetic enzyme with a compound to be tested; and
(e) comparing the activity of the branched chain amino acid biosynthetic enzyme that has been treated with a test compound to the activity of an untreated branched chain amino acid biosynthetic enzyme,

thereby selecting compounds with potential for inhibitory activity.